

CLAIMS

1. A method for determining a target nucleic acid sequence, wherein the target nucleic acid sequence is comprised in a preparation comprising a non-target nucleic acid sequence, the target nucleic acid sequence and the non-target nucleic acid sequence each having a first region of common sequence upstream of a first region of dissimilar sequence upstream of a second region of dissimilar sequence, the method comprising:
 - (a) contacting the preparation with a blocking oligonucleotide complementary to at least a portion of the first region of dissimilar sequence of the non-target nucleic acid sequence, under conditions to hybridise the blocking oligonucleotide thereto;
 - (b) contacting the preparation with a sequencing primer complementary to at least a portion of the first region of common sequence, under conditions to hybridise the primer to the target nucleic acid sequence; and
 - (c) subjecting the preparation to a sequencing reaction, such that the sequencing reaction proceeds into the second region of dissimilar sequence of the target nucleic acid sequence, thereby determining at least the second region of dissimilar sequence of the target nucleic acid sequence;and wherein the blocking oligonucleotide blocks the sequencing reaction at least from proceeding into the second region of dissimilar sequence of the non-target nucleic acid sequence.
2. A method according to claim 1, wherein the target nucleic acid sequence and the non-target nucleic acid sequence each have a second region of common sequence which lies between the first and second regions of dissimilar sequence.
3. A method according to claim 1 or claim 2, wherein step (a) further comprises a step of contacting the preparation with a terminator nucleotide,

under conditions to incorporate the terminator nucleotide into the blocking oligonucleotide hybridised to the non-target nucleic acid sequence.

4. A method according to claim 3, wherein the terminator nucleotide is a dideoxy nucleotide.
5. A method according to any preceding claim, wherein hybridisation of the blocking oligonucleotide to the non-target nucleic acid sequence is capable of inhibiting primer binding to the non-target nucleic acid sequence.
6. A method according to any of claims 1 to 4, wherein hybridisation of the blocking oligonucleotide to the non-target nucleic acid sequence is capable of inhibiting extension of the sequencing primer hybridised to the non-target nucleic acid sequence.
7. A method according to any preceding claim, wherein step (a) further comprises contacting the preparation with a cleavage agent which recognises a double-stranded recognition sequence comprising at least a part of the sequence of the blocking oligonucleotide, under conditions to cleave the non-target nucleic acid sequence.
8. A method according to claim 7, wherein step (a) comprises contacting the preparation with the blocking oligonucleotide, subjecting the preparation to a polymerisation reaction, under conditions to extend the blocking oligonucleotide hybridised to the non-target nucleic acid sequence, and contacting the preparation with the cleavage agent, under conditions to cleave the non-target nucleic acid sequence within the second region of common sequence.
9. A method according to claim 7 or claim 8, wherein the cleavage agent comprises a restriction endonuclease.

10. A method according to claim 9, wherein the restriction endonuclease recognises a recognition sequence comprising at least a part of the first region of dissimilar sequence of the non-target nucleic acid sequence.
11. A method according to claim 9 or claim 10, wherein the restriction endonuclease recognises a recognition sequence comprising at least a part of the second region of common sequence.
12. A method according to claim 7 or claim 8, wherein the cleavage agent comprises a chemical cleavage agent.
13. A method according to any of claims 3 to 12, wherein the terminator nucleotide is capable of covalently cross-linking the blocking oligonucleotide to the non-target nucleic acid.
14. A method according to any preceding claim, wherein the second region of dissimilar sequence comprises a single nucleotide.
15. A method according to any preceding claim, wherein the first region of dissimilar sequence comprises a single nucleotide.
16. A method according to any preceding claim, wherein the sequencing reaction comprises a method of sequencing based on the detection of the release of pyrophosphate.
17. A method according to claim 16, wherein the sequencing reaction comprises pyrosequencing.
18. A method according to any preceding claim, wherein the preparation comprises DNA derived from two or more individuals.

19. A method for determining a plurality of target nucleic acid sequences, wherein the plurality of target nucleic acid sequences is comprised in a preparation further comprising a plurality of corresponding non-target nucleic acid sequences, each target nucleic acid sequence in the preparation corresponds to one or more corresponding non-target nucleic acid sequences in the preparation, each target nucleic acid sequence and each corresponding non-target nucleic acid sequence has a first region of common sequence upstream of a first region of dissimilar sequence upstream of a second region of dissimilar sequence, the first region of common sequence of each target nucleic acid sequence is the same as the first region of common sequence of its corresponding non-target nucleic acid sequences, the first region of dissimilar sequence of each target nucleic acid sequence is different to the first region of dissimilar sequence of its corresponding non-target nucleic acid sequences, the second region of dissimilar sequence of each target nucleic acid sequence is different to the second region of dissimilar sequence of its corresponding non-target nucleic acid sequences, which method comprises:
- (a) contacting the preparation with a plurality of blocking oligonucleotides wherein each blocking oligonucleotide is complementary to at least a portion of the first region of dissimilar sequence of a non-target nucleic acid sequence, under conditions to hybridise the blocking oligonucleotide thereto;
 - (b) contacting the preparation with a plurality of sequencing primers, wherein each primer is complementary to at least a portion of the first region of common sequence of a target nucleic acid sequence and its corresponding non-target nucleic acid sequence, under conditions to hybridise the primer thereto; and
 - (c) subjecting the preparation to a sequencing reaction, such that the sequencing reaction proceeds into the second region of dissimilar sequence

of the target nucleic acid sequences, thereby determining at least the second region of dissimilar sequence of each target nucleic acid sequence; and wherein the blocking oligonucleotides block the sequencing reaction at least from proceeding into the second region of dissimilar sequence of each corresponding non-target nucleic acid sequence.

20. A method according to any preceding claim, wherein the target nucleic acid sequence and the non-target nucleic acid sequence comprise one or more further regions of dissimilar sequence downstream of the second region of dissimilar sequence.
21. A method for determining the haplotype of a subject from a sample comprising DNA from the subject, comprising a method as defined in any preceding claim, wherein the preparation comprises the sample, the target nucleic acid sequence comprises a locus on a first chromosome of a pair of chromosomes, the non-target nucleic acid sequence comprises the corresponding locus on the second chromosome of the pair, the locus comprising two or more single nucleotide polymorphisms for which the subject is heterozygous, wherein the sequencing reaction is conducted to determine the sequence of the locus on the first chromosome of the pair thereby determining the haplotype of the subject.
22. A method according to claim 21, where the locus comprises a human Class I or Class II HLA gene.
23. Use of pyrosequencing for determining the haplotype of a subject from a sample comprising DNA from the subject, wherein pyrosequencing is used to sequence a target locus on a first chromosome of a pair, the target locus comprising two or more single nucleotide polymorphisms, the corresponding locus on the second chromosome of the pair being blocked from

sequencing by a blocking oligonucleotide hybridised to the second chromosome.

24. Use according to claim 21, wherein the blocking oligonucleotide is hybridised to a region of the corresponding locus on the second chromosome which comprises a single nucleotide polymorphism.
25. A kit for determining one or more target nucleic acid sequences, wherein the one or more target nucleic acid sequences is comprised in a preparation comprising one or more non-target nucleic acid sequences, the one or more target nucleic acid sequences and the one or more non-target nucleic acid sequences each having a first region of common sequence upstream of a first region of dissimilar sequence upstream of a second region of dissimilar sequence, which kit comprises one or more blocking oligonucleotides complementary to at least a portion of the first region of dissimilar sequence of the one or more non-target nucleic acid sequences and one or more sequencing primers complementary to at least a portion of the first region of common sequence.
26. A kit according to claim 25, which further comprises one or more terminator nucleotides.
27. A kit according to claim 26, wherein the terminator nucleotide comprises a dideoxy nucleotide.
28. A kit according to claim 27, wherein the kit includes dideoxy-ATP, dideoxy-CTP, dideoxy-GTP and/or dideoxy-TTP.
29. A kit according to any of claims 25 to 28, further comprising deoxy-ATP, deoxy-CTP, deoxy-GTP, deoxy-TTP, a DNA polymerase, ATP sulfurylase, firefly luciferase and/or a nucleotide-degrading enzyme.